TRANSCAPILLARY MIGRATION OF HEAVY WATER AND THIOCYANATE ION IN THE PULMONARY CIRCULATION OF NORMAL SUBJECTS AND PATIENTS WITH CONGESTIVE HEART FAILURE [1, 2, 3]

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(Submitted for publication August 5, 1954; accepted September 8, 1954)

During recent years numerous investigations have been concerned with the rates of transfer or exchange of various substances across capillary beds (1–6). These studies, by analyzing curves of disappearance over several minutes from arterial plasma of intravenously injected tracer substances, have provided some information relative to all capillary beds combined. However, this type of experiment has not permitted a close analysis of the behavior of a substance during its first passage through a single organ capillary bed.

Recently developed techniques (7–9) now permit the clinical investigator to examine the phenomena of transcapillary exchange of water and other diffusible substances in local vascular areas. This study was undertaken in an attempt to determine *in vivo* the diffusion characteristics of the pulmonary capillary bed in man with regard to deuterium oxide and an electrolyte, thiocyanate ion.

Seven normal subjects and seven patients with pulmonary congestion due to heart failure were studied. The resulting data relating to the pulmonary capillaries were then compared to those previously obtained using similar tracer substances in the capillaries of the human forearm (7).

[1] Presented in part at the annual meeting of the American Federation for Clinical Research, Atlantic City, New Jersey, May 2, 1954.

[2] Supported in part by the Squibb Institute for Medical Research, New Brunswick, New Jersey and the Irwin Neisler Co., Decatur, Illinois.

[3] Sponsored in part by the Veterans Administration. Published with approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

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METHODS

A mixture of a non-diffusible substance (T-1824 or Evans blue dye) and substances whose diffusion is to be studied (deuterium oxide and thiocyanate ion) is injected into an afferent vessel of the organ under study. By sampling continuously from an efferent vessel for the period of the first circulation, the transcapillary exchange pattern can be determined.

The method is based on the principle that the relative concentrations of a mixture of diffusible and non-diffusible substances will be changed after passage through a capillary bed. The concentration of the non-diffusible substances in the effluent blood provides an index of the concentration of diffusible substance to be expected if none of the latter had been lost through the capillary walls. The difference between this expected concentration of the diffusible substance and that experimentally determined is a measure of the transcapillary loss (7).

A mixture usually consisting of 10 cc. of deuterium oxide, 10 cc. of 5 per cent sodium thiocyanate and 7 cc. of 0.5 per cent T-1824 dye was mixed well under sterile precautions. Approximately 20 cc. of this mixture was then injected rapidly from a calibrated syringe through an eighteen gauge needle usually into a basilic vein, although in four cases the injection was made through a cardiac catheter into the main pulmonary artery. Samples of blood were collected from the femoral artery through a fifteen gauge needle connected to a short piece of plastic tubing. Continuous sampling was carried out from the beginning to sixty seconds after injection. The blood was collected in paraffined test tubes containing one drop of dried heparin solution. The injection was accompanied by a verbal signal to a second assistant who began to call off second intervals from a stopwatch. The end of the plastic tubing was then moved from tube to tube at 2-second intervals until the timed collection period was completed. Immediately following the collection of samples the tubes were corked and the blood mixed with the heparin by gentle inversion. The samples were centrifuged and the supernatant plasma removed and analyzed for T-1824, SCN, and D_2O concentrations. A sample of the original or injected mixture was analyzed similarly.

The Evans blue concentrations were read in terms of optical density directly in a Coleman junior spectropho-

tometer. Thiocyanate was determined by the standard ferric nitrate method (10). The D_2O concentrations were determined by a spectrophotometric method based on the emission spectra of atomic hydrogen and deuterium in a high frequency electrodeless discharge (11). This method is capable of measuring concentrations of deuterium as low as 1 part in 6500 with an accuracy of 5 per cent and with correspondingly greater accuracy at the high concentrations measured in these experiments. The pulmonary blood flow for each patient was calculated from the T-1824 dye curve according to the method of Hamilton, Moore, Kinsman, and Spurling (12). The pulmonary water flow was calculated from the plasma flow by correcting for the hematocrit and the known water content of human plasma and red cells (7). The concentrations of D_2O and SCN to be expected, had there been no transcapillary diffusion, were calculated from the formula reported elsewhere (7). The formula for SCN was modified for incomplete penetration of red cells as determined by a separate *in vitro* determination. The penetration was found to be 70 rather than 100 per cent, and hence the hematocrit was multiplied by 0.7 in calculating the expected concentrations of thiocyanate.

The difference between these expected concentrations and the measured concentrations was expressed as per cent of expected concentration and plotted against time for each patient. The extravascular D_2O space was calculated according to a method to be discussed later.

RESULTS

Deuterium oxide—normal subjects

In the normal subjects (Table I) the initial loss of D_2O in the samples averaged 42 per cent of the expected concentrations. These values were obtained at a point on the ascending limb of the dye curve where the concentrations were sufficient to insure accurate measurement. This loss decreased rapidly and the expected and observed concentrations soon approached each other. In fact, within

TABLE I

Loss of deuterium oxide from the capillaries of the lung in normal subjects

Subject	Time after injection (*seconds*)	Hematocrit	T-1824 conc. in injecta (*opt. dens.*)	D_2O conc. in injecta (%)	T-1824 conc. (*opt. dens.*)	Expected D_2O concentration C_e (%)	Measured D_2O concentration C_0 (%)	Per cent loss D_2O	Slope loss *vs.* time curve (°)	Appearance time of T-1824 (*sec.*)	100% loss time (by extrapolation)
R. P.*	16	32	14.35	70.40	.280	1.02	.56	45			
	22				.590	2.14	1.96	8			
	24				.546	1.98	2.00	− 1	70	12	8
	28	32			.338	1.23	1.40	−14			
F. H.	25	41	15.65	66.20	.380	1.07	.64	40			
	31				.724	2.04	1.88	8			
	35	42			.448	1.27	1.91	−51	78	18	20
	39	42			.184	.51	.99	−93			
C. G.	19	41	21.85	51.60	.676	1.08	.69	36			
	21				.736	1.17	.87	26			
	23				.608	.96	.95	1	77	10	10
	29	41			.112	.16	.30	−85			
W. W.	12	42	23.10	50.50	.244	.35	.16	54			
	14				.430	.62	.36	42			
	16				.470	.68	.52	24	81	9	9
	20	42			.222	.32	.48	−50			
G. Y.	17	45	26.25	58.50	.470	.66	.39	41			
	19				.700	.99	.65	35			
	21				.658	.94	.76	19	78	11	12
	25	45			.344	.49	.58	−20			
S. P.	18	44	26.00	58.64	.560	.81	.45	45			
	20				.742	1.08	.67	38			
	22				.480	.70	.73	− 5	83	13	16
	24	44			.264	.38	.52	−37			
M. E.	11	36	74.50	36.14	.640	.22	.14	38			
	13				1.270	.44	.36	18			
	15				.996	.35	.38	−10	83	6	8
	17	36			.546	.19	.28	−48			
	19				.296	.10	.16	−59			

* Anemia but no evidence of pulmonary or cardiac disease.

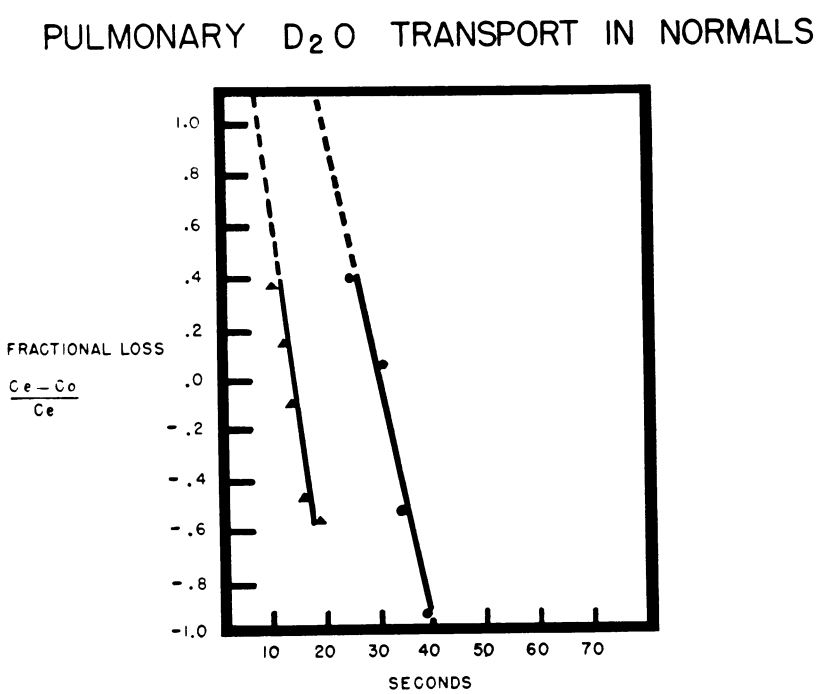

FIG. 1. CHART SHOWING FRACTIONAL LOSS D_2O *vs.* TIME FOR M. E. (DIAMONDS) AND F. H. (SQUARES) REPRESENTATIVE NORMAL SUBJECTS

Dotted line represents extrapolation. C_e represents expected concentrations based on T-1824 dye, C_o represents observed concentrations. Time is in seconds after dye appearance.

a few seconds after the peak dye concentration the collected samples contained more D_2O than the calculated expected concentrations. This implied that D_2O was returning to the circulation from some extravascular source. The curve representing the per cent loss of D_2O with respect to time seemed to be a straight line for each case and with approximately the same slope in all normal patients (Table I and Figure 1).

The curves of D_2O loss were extrapolated back to the appearance time of the dye T-1824. In all of the cases the extrapolation indicated a loss of 100 per cent D_2O at or near the appearance time (Table I and Figure 1).

Deuterium oxide—cardiac failure patients

In the patients with pulmonary congestion the slope of D_2O loss showed a similar straight line relationship in time (Figure 2). Most of these curves also extrapolated back to 100 per cent D_2O loss near appearance time. It can be seen, however, that the slope of these curves was less than in the normal subjects (Figure 2 and Table II). The average slope for the cardiac failure patients was 60 degrees as compared to 79 degrees for the normal subjects.

Calculation of pulmonary extravascular water space

Based on what seems to be a valid assumption that the D_2O exchange is complete in one circulation (see discussion) it is possible to calculate the size of the extravascular D_2O space for each patient: Let C_o = observed concentrations at time t of D_2O and C_e = expected concentration at time t of D_2O had there been no transcapillary diffusion, then

$$\int_{t_a}^{t_{equil.}} F\,(C_e - C_o)\,dt = \text{amount of } D_2O \text{ in extravascular space} = Q$$

where $t_{equil.}$ = time after appearance when $C_e = C_o$, and t_a = appearance time, and F = pulmonary water flow.

Since the concentration ($C_{equil.}$) of D_2O in the extravascular D_2O space at $t_{equil.}$ is

$$\frac{Q}{\text{Volume extravascular space}},$$

then by rearrangement:

$$\text{Volume extravascular space} = \frac{Q}{C_{equil.}}.$$

This volume can then be calculated from Q, obtained by integration of the curve of $C_e - C_o$ multiplied by the pulmonary water flow (calculated from the T-1824 curves), and $C_{equil.}$ which is the concentration when $C_e = C_o$. The volumes of the pulmonary extravascular space calculated by this method averaged 190 cc. (range 105 to 360 cc.) in the normal subjects and 290 cc. (range 113 to 330 cc.) in the patients with pulmonary congestion (Table III). When the volumes of the extravascular D_2O spaces in the normal subjects were plotted against the pulmonary blood flows (Figure 3), it appeared that the size of the space was a monotonic increasing function of the blood flow. This relationship did not hold, however, in the patients with pulmonary congestion.

Thiocyanate ion—normal subjects and cardiac failure patients

The thiocyanate pulmonary capillary diffusion pattern differed markedly from that of heavy water. In contrast to the large losses and constant diffusion pattern observed with the deuterium oxide, thiocyanate ion loss was negligible in the normals and only slightly higher in the patients with pulmonary congestion. No definite pattern of loss was discernible except for the relatively low values throughout the circulation period (Table IV).

DISCUSSION

The principle on which the method is based implies the use of a non-diffusible substance for comparison with the studied tracers. That Evans

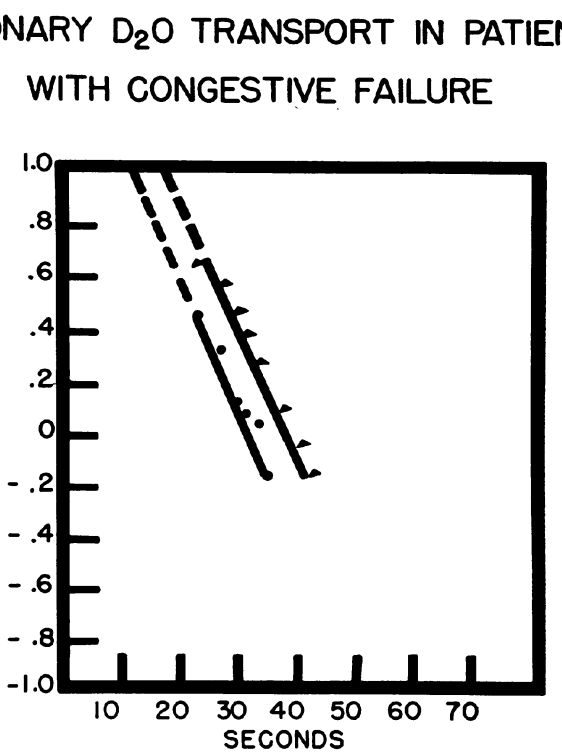

FIG. 2. CHART SHOWING FRACTIONAL LOSS D_2O *vs.* TIME FOR B. B. (CIRCLES) AND S. B. (TRIANGLES) REPRESENTATIVE PATIENTS WITH PULMONARY CONGESTION DUE TO HEART FAILURE

Dotted line represents extrapolation.

TABLE II

Loss of deuterium oxide from the capillaries of the lung in patients with pulmonary congestion due to heart failure

Patient and diagnosis	Time after injection (*sec.*)	Hematocrit	T-1824 conc. in injecta (*opt. dens.*)	D_2O conc. in injecta (%)	T-1824 concentration (*opt. dens.*)	Expected D_2O concentrations C_e (%)	Measured D_2O concentrations C_o (%)	Per cent loss D_2O	Slope of loss *vs.* time curve (°)	Appearance of time of T-1824 (*sec.*)	100% loss time (by extrapolation)
J. D.	30	37	38.10	46.70	.188	.16	.10	38			
Hypertensive heart	32				.258	.22	.16	30			
disease	36				.348	.30	.25	17	60	22	12
Congestive failure	38	37			.368	.32	.28	11			
	42				.318	.28	.32	−16			
R. F.	27		33.80	43.68	.512	.47	.32	32			
Acute myocardial	29	36			.598	.55	.43	22			
infarction	31				.558	.51	.44	15	67	14	14
Congestive failure	35				.402	.37	.40	− 7			
	39	36			.200	.19	.23	−22			
H. B.	41		32.00	47.09	.174	.16	.09	45			
Arteriosclerotic heart	45	45			.300	.28	.16	41			
disease	49				.384	.36	.22	38	46	33	24
Congestive failure	57				.460	.43	.34	21			
	61	45			.436	.41	.36	11			
	65				.400	.37	.38	− 2			
B. B.	24		33.80	53.70	.500	.59	.33	44			
Hypertensive heart	28	33			.780	.92	.64	31			
disease	30				.720	.85	.72	15	68	16	14
Congestive failure	32	33			.680	.80	.72	10			
	34				.528	.62	.60	4			
	36				.420	.49	.56	−13			
S. B.	24	25	32.40	49.75	.256	.31	.12	62			
Hypertensive heart	28				.580	.72	.32	56			
disease	30				.680	.85	.44	48			
Congestive failure	32				.680	.85	.52	38	68	18	19
	34	25			.616	.77	.56	27			
	36				.540	.67	.58	13			
	40				.428	.53	.54	− 2			
	42	25			.370	.46	.52	−13			
D. S.	55		32.40	46.52	.296	.30	.24	19			
Arteriosclerotic heart	57				.332	.33	.29	12			
disease	59	38			.388	.39	.33	15			
Congestive failure	61				.428	.43	.39	8	47	40	25
	63				.444	.44	.39	12			
	65				.444	.44	.44	1			
	67				.440	.44	.44	0			
	69				.408	.41	.40	2			
	71				.380	.38	.40	− 5			
	73	38			.352	.35	.38	− 8			
	75				.320	.32	.36	−13			
R. S.	23	49	34.50	45.89	.336	.27	.18	30			
Arteriosclerotic heart	25				.450	.36	.28	21			
disease	27				.444	.35	.30	15	67	18	10
Congestive failure	29	49			.390	.31	.30	3			
	37				.282	.24	.26	− 8			

blue dye is practically non-diffusible in one circulation through the normal lung seems to be a valid assumption (13, 14). Studies are now in progress to see if this is equally valid in heart failure, but the close agreement between cardiac outputs calculated from Evans blue dye curves and by the direct Fick method would seem to lend support to such an assumption.

The results obtained for deuterium oxide loss in the lung differed markedly from those previously reported for the forearm capillaries (7). In the forearm more than 95 per cent of the circulating D_2O is lost from the capillary bed throughout the first circulation period with minimal change in this loss during that time. By contrast, in the lungs initial losses were large but this was followed within a few seconds by significant gains of D_2O into the blood stream. This pattern observed in both normal subjects and congestive failure patients implied a relatively small extravascular tissue space in the lungs as compared with the forearm.

The estimation of the pulmonary extravascular tissue space in the patients studied provides credible values (average 190 cc. in normal subjects and 290 cc. in patients with congestive failure). These values seem reasonable in view of the known lung weights and the histologic consideration that the lung is composed for the most part of capillaries and air spaces, with very little extravascular tissue. The assumption on which the calculation of the lung extravascular space is based is

TABLE III

Estimated extravascular D_2O space in normal subjects and patients with pulmonary congestion due to heart failure

Normal subjects			Heart failure patients		
Subject	Pulmonary blood flow (*ml. sec.*)	Extravascular D_2O space (*cc.*)	Patient	Pulmonary blood flow (*ml. sec.*)	Extravascular D_2O space (*cc.*)
R. P.	80	105	J. D.	150	330
F. H.	90	110	R. F.	115	320
C. G.	80	180	H. B.	50	350
G. Y.	120	225	B. B.	70	255
S. P.	180	360	D. S.	50	113
W. W.	155	275	R. S.	143	310
M. E.	135	111			
	Average	190		Average	290

that there is complete exchange of circulating D_2O with extravascular water during one circulation. This assumption seems to be valid for when the curves of loss of D_2O in the lung are extrapolated backward they cross the 100 per cent loss axis at or very near the appearance time.

A comparison of thiocyanate losses from the forearm capillaries previously reported (*7*) with those now found in the lung capillaries reveals a marked difference in transcapillary diffusion patterns. The losses in the forearm, when recalculated correcting for the incomplete red blood cell penetration, average about 70 per cent, with a slight decrease in this loss during the circulation period. By contrast, the losses in the lung were less than 10 per cent in the normal subjects and

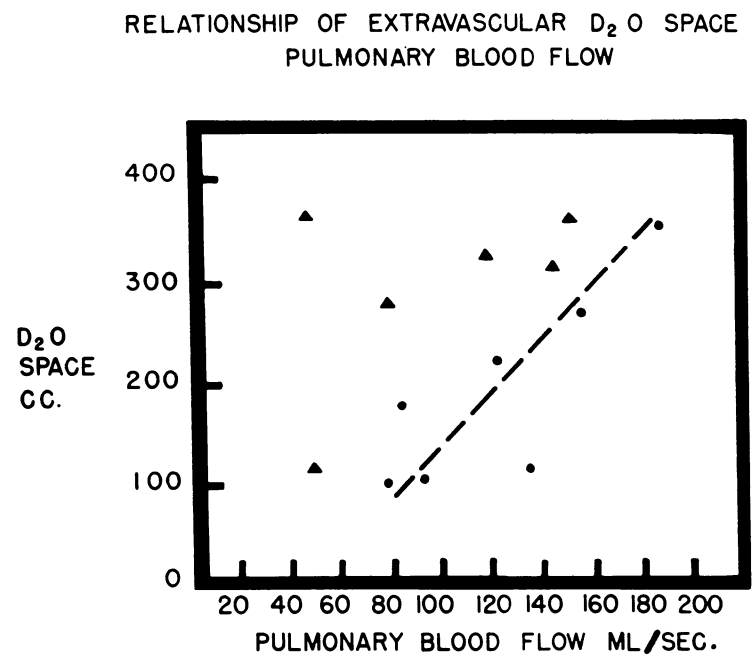

FIG. 3. CHART SHOWING PULMONARY EXTRAVASCULAR D_2O SPACE *vs.* PULMONARY BLOOD FLOW FOR NORMAL SUBJECTS (CIRCLES) AND PATIENTS WITH CONGESTIVE HEART FAILURE (TRIANGLES)

Dotted line represents apparent relationship in normal subjects. No relationship between size of space to blood flow is apparent in the patients with pulmonary congestion. Note also that all points representing extravascular D_2O space in congestive failure are above the line representing the normal subjects.

TABLE IV

Thiocyanate loss from the lung capillary bed in normal subjects and patients with pulmonary congestion due to heart failure

Normal subjects			Congestive failure patients		
Time after dye appearance (seconds)	Per cent loss of SCN (7 subjects) Average	S.D.	Time after dye appearance (seconds)	Per cent loss of SCN (7 patients) Average	S.D.
5	2.5	±7.4	5	8.5	±10.9
7	4.5	±4.1	7	14.5	±13.9
9	8.5	±5.9	9	11.4	± 3.7
11	9.3	±3.1	11	16.3	± 7.0
13	9.3	±7.4	13	11.7	± 5.3
			15	11.6	± 8.9
			17	9.7	± 4.6
			19	11.9	± 3.2
			21	12.3	± 4.8
			23	9.0	±11.6
			25	15.0	± 0.0
			27	10.7	± 4.5

less than 20 per cent in the patients with pulmonary congestion. Since the loss of thiocyanate is small *throughout* the circulation period it would seem to suggest an inherent difference in the permeability of the lung capillary walls to this ion as compared to the forearm. Should the small loss be due only to a very small interstitial space, it would be expected that the losses would be higher during the first part of the circulation rather than constantly small throughout. The slightly higher loss in the congested lung as compared with the normal lung can probably be explained on the basis of slight increase in permeability and/or slightly greater area available for diffusion. The relative impermeability of the lung capillaries to the electrolytes, Na^+ and I^- has been suggested by the work of other investigators (9), and seems, therefore, to be a characteristic of the pulmonary capillary bed.

The relation between the extravascular D_2O space and the pulmonary blood flow in the normal patients (Figure 3) suggests that the extravascular water space may be dependent upon the functioning capillary bed and may perhaps serve as an index of its size. Studies are now in progress in normal subjects to determine whether the extravascular D_2O space will change in the presence of induced variations in pulmonary blood flow.

SUMMARY

By employing a technique of injection into a basilic vein or pulmonary artery of a mixture of non-diffusible and diffusible substances and sampling during the period of first circulation from the femoral artery the following observations were made in man with regard to passage of deuterium oxide and thiocyanate ion through the pulmonary capillaries in seven normal subjects and seven patients with pulmonary congestion due to heart failure:

1. Deuterium oxide freely and rapidly passed through the pulmonary capillary walls.

2. When the transcapillary loss of D_2O was plotted against time, the curve obtained differed markedly from that found previously in the forearm (7) and indicated a small pulmonary extravascular tissue space.

3. Quantitative estimation of the extravascular water space of the lung indicated a mean value of 190 cc. in the normal subjects and of 290 cc. in the patients with congestive heart failure. In normal, but not in cardiac patients, the size of the pulmonary extravascular D_2O space appeared to be related to the pulmonary blood flow.

4. Thiocyanate ion in contrast to the previously observed large transfer through forearm capillaries left the pulmonary capillaries in only small amounts in the normal patients and in only slightly greater amounts in the patients with congestive failure. An analysis of these findings suggests a relative impermeability of the lung capillaries as compared to the forearm capillaries with respect to this ion.

ACKNOWLEDGMENT

The authors gratefully acknowledge the valuable technical assistance of Miss Jean Pietras and Miss Margaret Selgin.

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